

Stable isotopes reveal regional heterogeneity in the pre-breeding distribution and diets of sympatrically breeding *Pygoscelis* spp. penguins

Michael J. Polito^{1,*}, Heather J. Lynch², Ron Naveen³, Steven D. Emslie¹

¹Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 South College Road, Wilmington, North Carolina 28403, USA

²Department of Biology, 3237 Biology-Psychology Building, University of Maryland, College Park, Maryland 20742, USA

³Oceanites Inc., PO Box 15259, Chevy Chase, Maryland 20825, USA

ABSTRACT: While the foraging ecology of the Adélie penguin *Pygoscelis adeliae* and gentoo penguin *P. papua* has been well studied, little is known on the distribution and diet of these species outside the breeding season. In the present study we used stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analyses of eggshells to examine the pre-breeding diets and foraging habitats of female Adélie and gentoo penguins from 23 breeding colonies along the eastern and western Antarctic Peninsula (AP), South Shetland Islands, and South Orkney Islands, in 2006. Adélie penguin eggshells from the eastern AP, South Shetland Islands, and South Orkney Islands shared similar isotopic signatures and were significantly lower in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than eggshells from birds breeding along the western AP. This result suggests that Adélie penguin populations that are geographically separated during the breeding season by the Adélie 'gap,' a 400 km region in the western AP devoid of breeding Adélie penguins, also inhabit geographically distinct habitats during the late winter prior to the breeding season. To a lesser degree, gentoo penguin eggshell isotope values also varied across breeding colonies, which likely reflects local scale variation in their near-shore foraging grounds. Furthermore, unlike the breeding period when krill (primarily *Euphausia superba*) dominates penguin diets in these regions, our findings suggest that fish and/or other high trophic-level prey species comprise a significant portion (46.8 to 62.9%) of female Adélie and gentoo penguin diets prior to breeding.

KEY WORDS: Stable isotope analysis · Adélie penguin · Gentoo penguin · *Pygoscelis adeliae* · *Pygoscelis papua* · Distribution · Diet

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INTRODUCTION

Studies of the foraging ecology of penguins have been commonly used as a cost-efficient method to monitor the Antarctic marine ecosystem (Trivelpiece et al. 1990, Reid & Croxall 2001). These studies are of increased relevance in the Antarctic Peninsula (AP), where recent changes in climate, sea ice conditions, and the abundance of Antarctic krill *Euphausia superba* have been suggested to be responsible for contrasting population changes in *Pygoscelis* spp. penguins (Fraser & Hofmann 2003, Forcada et al. 2006, Hinke et al. 2007). For example, there is evidence of a

widespread and long-term decrease in Adélie penguin *P. adeliae* populations in the AP, while gentoo penguin *P. papua* populations are stable or increasing, with recent expansions in the southernmost part of their breeding range (Lynch et al. 2008).

While the foraging ecology of Adélie and gentoo penguins has been well studied, research has been limited to a few breeding colonies throughout this large region. Adélie penguins breed in the AP region from Marguerite Bay to Anvers Island along the western AP and in the eastern AP, South Shetland Islands, and South Orkney Islands (Fig. 1a). Gentoo penguins breed between the Yalour Islands and Trinity Island

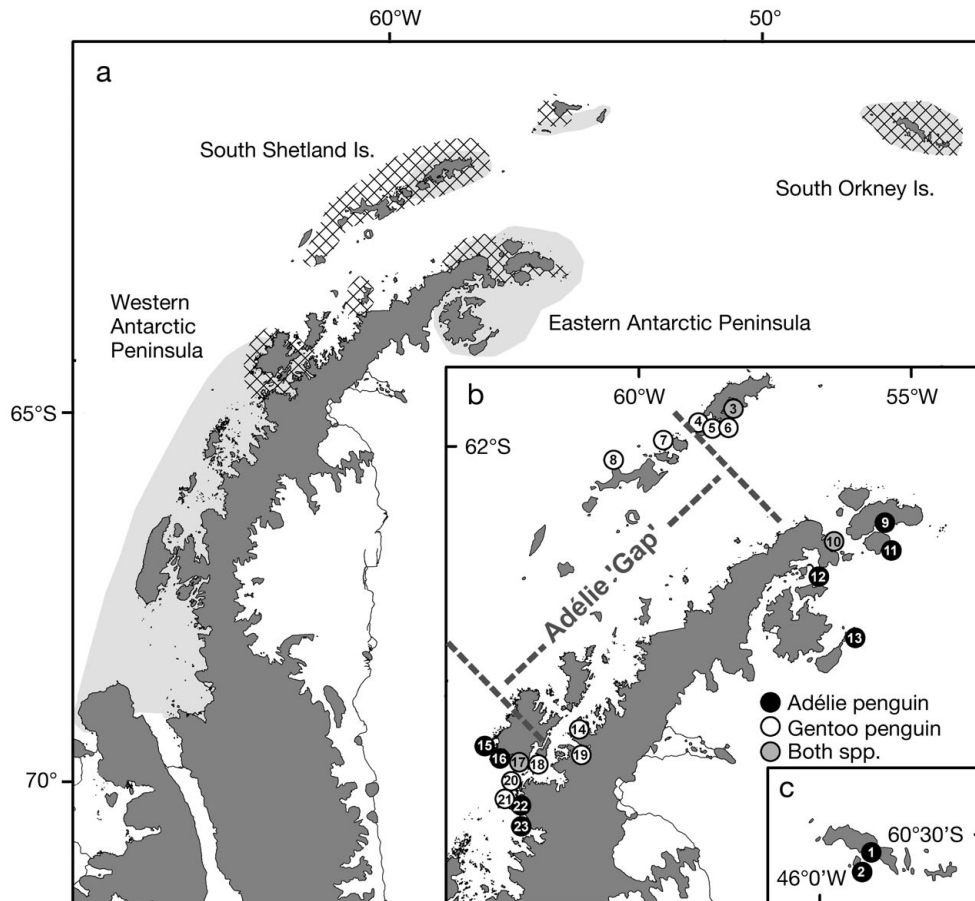


Fig. 1. *Pygoscelis adeliae* and *P. papua*. (a) Summer breeding distributions of Adélie penguins (light shading) and gentoo penguins (cross hatch), and (b,c) location of 23 breeding colonies sampled for eggshells in the South Orkney Islands, South Shetland Islands, eastern and western Antarctic Peninsula during austral summer of 2006–07. Breeding distributions adapted from data in Woehler (1993) and Lynch et al. (2008). Note the conspicuous gap in the breeding range of Adélie penguins along the western Antarctic Peninsula and South Shetlands Islands (the Adélie gap; dashed line). See Tables 2 & 3 for the corresponding site names for each of the 23 breeding colonies

along the Western AP, in a few locations in the eastern AP, and throughout much of the South Shetland and South Orkney Islands (Fig. 1a) (Woehler 1993, Lynch et al. 2008). During the breeding season, Adélie penguins in the AP primarily feed on krill and small amounts of fish, while gentoo penguin diets are more flexible and contain variable amounts of krill and fish (Volkman et al. 1980, Karnovsky 1997, Miller et al. 2009). While these species' distributions overlap, it is thought that during breeding, gentoo penguins forage preferentially inshore, in benthic habitats, while Adélie penguins often forage offshore, in pelagic waters (Trivelpiece et al. 1987, Kokubun et al. 2010, Wilson 2010).

In contrast, little is known about the diets and distributions of these species outside of the breeding season. The few existing studies of winter diets of Adélie and gentoo penguins in the AP suggest that high trophic-level prey, such as fish and squid, are dominant prey items (Jablonski 1985, Ainley et al. 1992,

Coria et al. 2000). During winter, Adélie penguins are concentrated in the pack ice, while gentoo penguins are generally non-migratory and remain near their breeding colonies year-round (Fraser et al. 1992, Wilson et al. 1998, Tanton et al. 2004). While the exact wintering distributions of Adélie penguin populations that breed in the AP are unknown, high numbers have been observed in the winter pack ice east of the AP in the Weddell Sea and west of the AP in the Bellingshausen Sea (Ainley et al. 1992, Fraser et al. 1992, Fraser & Trivelpiece 1996, Chapman et al. 2004, Ribic et al. 2008). This finding, along with the distinctive gap in the breeding range of Adélie penguins between Anvers Islands in the south and King George Island and the Eastern AP in the north (the Adélie 'gap', Fig. 1b) (Woehler 1993), has led some to hypothesize that Adélie penguins in the AP may comprise 2 distinct populations: one that breeds from Anvers Island to the south along the western AP and winters in the Bellingshausen Sea, and another that breeds in the

South Orkney Islands, South Shetland Islands, and eastern AP, and winters in the Weddell Sea (Fraser et al. 1992, Fraser & Trivelpiece 1996, Trivelpiece & Fraser 1996). However, there is currently little evidence to support or reject this hypothesis, and to our knowledge only 1 published study has tracked breeding Adélie penguins in this region from their colony to their winter habitats (Dunn et al. in press).

The use of naturally occurring stable isotopes to describe the foraging ecology of penguins has become commonplace, as the stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in animal tissues are largely determined by isotopic abundances in an animal's food web (Ainley et al. 2003, Cherel & Hobson 2007, Cherel 2008, Tierney et al. 2008, Polito et al. 2009). Nitrogen isotope values are commonly used to infer trophic level and diets, while carbon values help trace vertical trends in habitat use (benthic vs. pelagic) as well as the geographic location of foraging habitats (Quillfeldt et al. 2005, Cherel & Hobson 2007, Quillfeldt et al. 2010). Moreover, this technique shows great promise in elucidating the diets and foraging distributions of seabirds outside the breeding season when birds are away from their breeding colony (Quillfeldt et al. 2005, Cherel et al. 2007, Phillips et al. 2009, Polito et al. 2009).

Previous studies on quail *Coturnix japonica* and ostrich *Struthio camelus* have found that stable isotope analysis of eggshell provides information on diet during a brief period prior to breeding, as eggshell components are derived from plasma through the metabolism of recently assimilated foods (Simkiss & Tyler 1958, Hobson 1995, Johnson 1995, Johnson et al. 1998). However, this is likely not the case in birds that utilize significant amounts of stored nutrient reserves during egg-laying, such as penguins (Astheimer & Grau 1985, Hobson 1995). Female Adélie penguins fast for up to 2 wk prior to egg-laying, and approximately 75% of the energy content of the clutch is deposited after females have begun their fast (Astheimer & Grau 1985). Female gentoo penguins also fast before egg-laying, losing 12% of their total body mass at a rate of 85 g d^{-1} prior to laying their first egg (Trivelpiece & Trivelpiece 1990). Therefore, it is likely that the isotopic values of Adélie penguin and to a lesser extent gentoo penguin eggshells reflect an integration of dietary information during late-winter foraging prior to the penguins' return to their breeding colonies and/or the onset of the egg-laying fast.

In the present study we examined the pre-breeding diets and foraging habitats of female Adélie and gentoo penguins using stable isotope analysis of eggshells collected from 23 breeding colonies along the eastern and western AP, South Shetland Islands, and South Orkney Islands. Specifically, we tested the hypothesis put forward by Fraser et al. (1992) and others that Adélie penguin breeding populations, which are geogra-

phically separated by the Adélie gap, inhabit geographically distinct habitats outside of the breeding season. A recent analysis of the $\delta^{13}\text{C}$ signatures of pelagic primary producers highlighted isotopic differences between the 2 proposed Adélie penguin wintering areas in the Bellingshausen and Weddell Seas (Quillfeldt et al. 2010). Due to these differences, the isotopic signatures of Adélie penguin eggshells should allow for discrimination between populations using these 2 winter habitats prior to breeding. Secondly, we used an isotopic mixing model to quantify the diet composition of female Adélie and gentoo penguins prior to breeding to confirm the few existing studies of winter diets which suggest that high trophic-level prey are important components of diets during this time period (Jablonski 1985, Ainley et al. 1992, Coria et al. 2000). Lastly, we examined how sensitive mixing-model estimates of penguin diet compositions are to variations in isotopic discrimination factors between wild and captive penguin populations.

MATERIALS AND METHODS

Study area and sample collection. We collected eggshell samples from 23 Adélie and gentoo penguin breeding colonies along the AP (roughly 60 to 66°S ; Fig. 1b,c). This area defines the range of overlap for these 2 species and naturally clusters into 4 regions: the South Orkney Islands, the South Shetland Islands, the western AP, and the eastern AP (Fig. 1a). From November 2006 to February 2007, eggshell samples were collected by researchers based on the tour ship 'National Geographic Endeavour' and several land-based scientific stations. At each breeding colony we opportunistically collected 8 to 20 eggshells from hatched, depredated, addled, or infertile eggs of each species. When sampling in mixed-species colonies, we collected only eggshells that could be identified to species when they were found in close proximity to nests in non-mixed sections of colonies. We cleaned eggshells of organic debris in the field using a toothbrush and water; then dried and stored samples at room temperature prior to further preparation for isotopic analysis.

Penguin prey items were collected during trawls conducted in the vicinity of the AP and South Shetland and South Orkney Islands by the US Antarctic Marine Living Resource Program (AMLR) during the austral summers of 2005–06 to 2008–09. Sampled prey species were representative of the 2 major components of Adélie and gentoo penguin diets in the study area: krill *Euphausia superba* ($n = 20$) and fish (Adélie penguin: *Pleuragramma antarcticum*, $n = 30$; gentoo penguin: *Lepidonotothen squamifrons*, $n = 10$) (Volkman et al. 1980, Karnovsky 1997, Miller et al. 2009). Whole krill and fish samples were kept frozen prior to analysis.

Sample preparation and isotopic analysis. Eggshell membranes were removed using a Dremel tool with a sanding attachment. Eggshells were then rinsed in distilled water, cleaned of any remaining surface debris, and then ground to a powder using an analytical mill. Isotope values of the organic matrix of penguin eggshells were obtained after the removal of carbonate by dissolving ~10 mg of cleaned eggshell in a silver capsule through titration with five 20 μ l aliquots of 6 N HCl. Acidified samples were stored at room temperature under a fume hood for 24 h, then dried for at least 48 h in an oven at 60°C. Acidified samples were not rinsed prior to drying and isotopic analysis to avoid biasing $\delta^{15}\text{N}$ values (Jacob et al. 2005). The mean C:N value of acidified eggshells was 3.8 ± 0.2 , which closely approached the assumed C:N value for pure protein of 3.7 (Fry et al. 2003).

Whole krill and fish samples were homogenized and then dried for 48 h in an oven at 60°C. Lipids were then extracted from whole fish and krill samples using a Soxhlet apparatus with a 1:1, petroleum-ether: ethyl-ether solvent mixture for 8 h (Seminoff et al. 2007). Lipid-extracted prey items were not acidified prior to isotopic analysis. Approximately 0.5 mg of each eggshell, krill and fish sample was loaded into tin cups for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis.

Eggshell, krill and fish tissues were flash-combusted (Costech ECS4010 elemental analyzer) and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ through an interfaced Thermo Delta V Plus continuous-flow stable isotope ratio mass spectrometer (CFIRMS). Raw δ values were normalized on a 2-point scale using depleted and enriched glutamic acid reference materials USGS-40 and USGS-41. Sample precision was 0.1‰ and 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively.

Stable isotope abundances are expressed in δ notation in per mille units (‰), according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$. The R_{standard} values were based on the Vienna PeeDee Belemnite (VPDB) for ^{13}C and atmospheric N_2 for ^{15}N .

Statistical and dietary analysis. We grouped eggshell samples by species and region and used separate, univariate ANOVA with 1 factor and 7 levels (1 level for each species–region combination as no gentoo penguin eggshells were collected from the South Orkney Islands). We also applied Tukey-Kramer multiple comparison tests to examine pair-wise differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of eggshells across species and regions. In addition, we examined variation in the isotopic values of eggshells within and across regions in the AP for each species using separate, univariate

ANOVAs with 1 factor (breeding colony) and Tukey-Kramer multiple comparison tests. To complement the univariate analyses, we used multivariate discriminant analyses with a jackknife (leave-one-out) classification to examine our ability to differentiate between Adélie and gentoo penguin samples and to assign Adélie and gentoo penguins from each region into discrete groups that share similar pre-breeding diets and foraging habitats based on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of their eggshells.

We examined variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of common Adélie and gentoo penguin prey species using univariate ANOVAs. We quantified the contribution of krill *Euphausia superba* and fish (*Pleuragramma antarcticum* for Adélie penguins and *Lepidonotothen squamifrons* for gentoo penguins) to the diets of pre-breeding female penguins in each region by using a single-isotope ($\delta^{15}\text{N}$), 2-source linear mixing model (Phillips & Gregg 2001). Model results provide standard errors and confidence intervals for source proportion estimates that account for the observed variability in the isotopic signatures for the sources as well as the mixture. We corrected eggshell values for dietary isotopic fractionation using a $\delta^{15}\text{N}$ discrimination factor of +1.8‰ derived from a captive population of gentoo penguins (Polito et al. 2009). The resulting mixing model-based diet composition estimates were compared across species–region combinations with t -tests calculated using the Satterthwaite (1946) approximation for the degrees of freedom. As discrimination factors can vary by species and/or the elemental and isotopic composition of an animal's diet, we performed a sensitivity analysis (+0.8‰ to +2.8‰ by increments of 0.5‰) in order to examine how sensitive our estimates of female pre-breeding diets might be to possible differences between $\delta^{15}\text{N}$ discrimination factors in the wild and those estimated in a captive population (Post 2002, Cherel et al. 2005a,b, Caut et al. 2008a, Robbins et al. 2010).

Data were examined for normality and equal variance. All tests were 2-tailed, and significance was assumed at the 0.05 level. Statistical calculations were performed using SAS (version 9.1) and SPSS (version 16.0). All means are presented \pm SD, except diet composition estimates resulting from our linear mixing model, which are presented \pm SE.

RESULTS

Inter-specific and spatial variation in eggshell isotopic values (Figs. 2 & 3)

We measured significant differences in eggshell $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values across species–region combinations ($\delta^{15}\text{N}$: $F_{6,375} = 13.090$, $p < 0.001$; $\delta^{13}\text{C}$: $F_{6,375} =$

72.074, $p < 0.001$). Post hoc Tukey comparisons suggested that, in general, Adélie penguins have lower eggshell $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than gentoo penguins, except in the western AP, where Adélie and gentoo penguin eggshells have similar $\delta^{15}\text{N}$ values, and in the eastern AP, where these 2 species have similar $\delta^{13}\text{C}$ values (Table 1). Discriminant analysis found that isotopic values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) significantly differentiated eggshells of Adélie and gentoo penguins (Wilks' lambda = 0.647, $\chi^2 = 161.958$, $df = 2$, $p < 0.001$). Overall correct assignment after jackknife validation was 79.2% (77.2% of Adélie penguin and 81.3% of gentoo penguin eggshells). The ability of the resulting discriminant function to correctly assign eggshell samples to species varied by region. Adélie penguin eggshells were correctly assigned to species with an accuracy of 100, 100, 87.8, and 46.0% for eggshells from the South Orkney Islands, South Shetland Islands, eastern AP, and western AP, respectively. Gentoo penguin eggshells were correctly assigned to species with an accuracy of 84.1, 21.4, and 88.7% for eggshells from the South Shetland Islands, eastern AP, and western AP, respectively.

While we found few differences in Adélie penguin eggshell $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values across breeding colonies in the South Orkney Islands, South Shetland Islands, and the eastern AP, differences between eggshells from these 3 regions and the western AP were relatively more common (Table 2; $\delta^{15}\text{N}$: $F_{12,193} = 8.966$, $p < 0.001$; $\delta^{13}\text{C}$: $F_{12,193} = 9.855$, $p < 0.001$). Adélie penguins breeding in the South Orkney Islands, South Shetland Islands, and eastern AP shared similar eggshell $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, and these were lower than the isotopic values of eggshells from Adélie penguins breeding in the western AP (Fig. 2a). While it showed statistical significance, discriminant analysis based on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values did a poor job of differentiating between eggshells of Adélie penguin breeding in the 4 sampled regions (Wilks' lambda = 0.574, $\chi^2 = 104.756$, $df = 6$, $p < 0.001$). Overall correct assignment after jackknife validation was 47.2% (Fig. 3a; 26.7, 66.7, 20.3, and 78.4% for eggshells from the South Orkney Islands, South Shetland Islands, eastern AP, and western AP, respectively). However, eggshell isotopic values were much better at discriminating between northern (South Orkney Islands, South Shetland Islands, and eastern AP) and southern (western AP) breeding colonies (Wilks' lambda = 0.596, $\chi^2 = 98.44$, $df = 2$, $p < 0.001$). Overall correct assignment after jackknife validation was 79.8% (Fig. 3c; 77.3% of the northern group and 81.8% of the southern group).

While there was some variation in the isotopic signatures of gentoo penguin eggshells at the breeding colony level, differences within and across regions in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were less common relative to the

differences observed in Adélie penguins (Table 3; $\delta^{15}\text{N}$: $F_{12,182} = 10.388$, $p < 0.001$; $\delta^{13}\text{C}$: $F_{12,182} = 16.362$, $p < 0.001$). Gentoo penguin eggshell $\delta^{15}\text{N}$ values did not differ across regions, while eggshell $\delta^{13}\text{C}$ values in gentoo penguins breeding in the eastern AP were lower than those breeding in the South Shetland Islands and the western AP (Fig. 2b). Similarly, while it showed statistical significance, discriminant analysis based on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values did a poor job of differentiating between eggshells of gentoo penguins breeding in the 3 sampled regions (Wilks' lambda = 0.763, $\chi^2 = 48.206$, $df = 4$, $p < 0.001$). Correct assignment after jackknife validation was 54.9% (Fig. 3b; 46.6, 78.6, and 60.0% for eggshells from the South Shetland Islands, eastern AP, and western AP, respectively). However, eggshell isotopic values were much better at discriminating between gentoo penguin colonies on the eastern and western (including the South Shetland Islands) sides of the AP (Wilks'

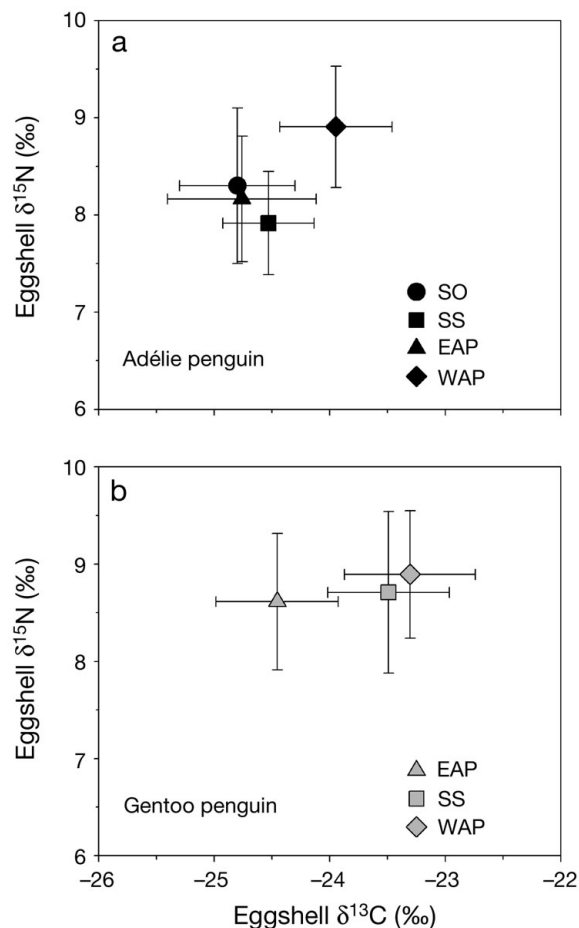


Fig. 2. *Pygoscelis adeliae* and *P. papua*. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ concentrations of (a) Adélie and (b) gentoo penguin eggshells collected from breeding colonies in the South Orkney Islands (SO), South Shetland Islands (SS), eastern Antarctic Peninsula (EAP), and western Antarctic Peninsula (WAP) during austral summer of 2006–07. Error bars represent SD

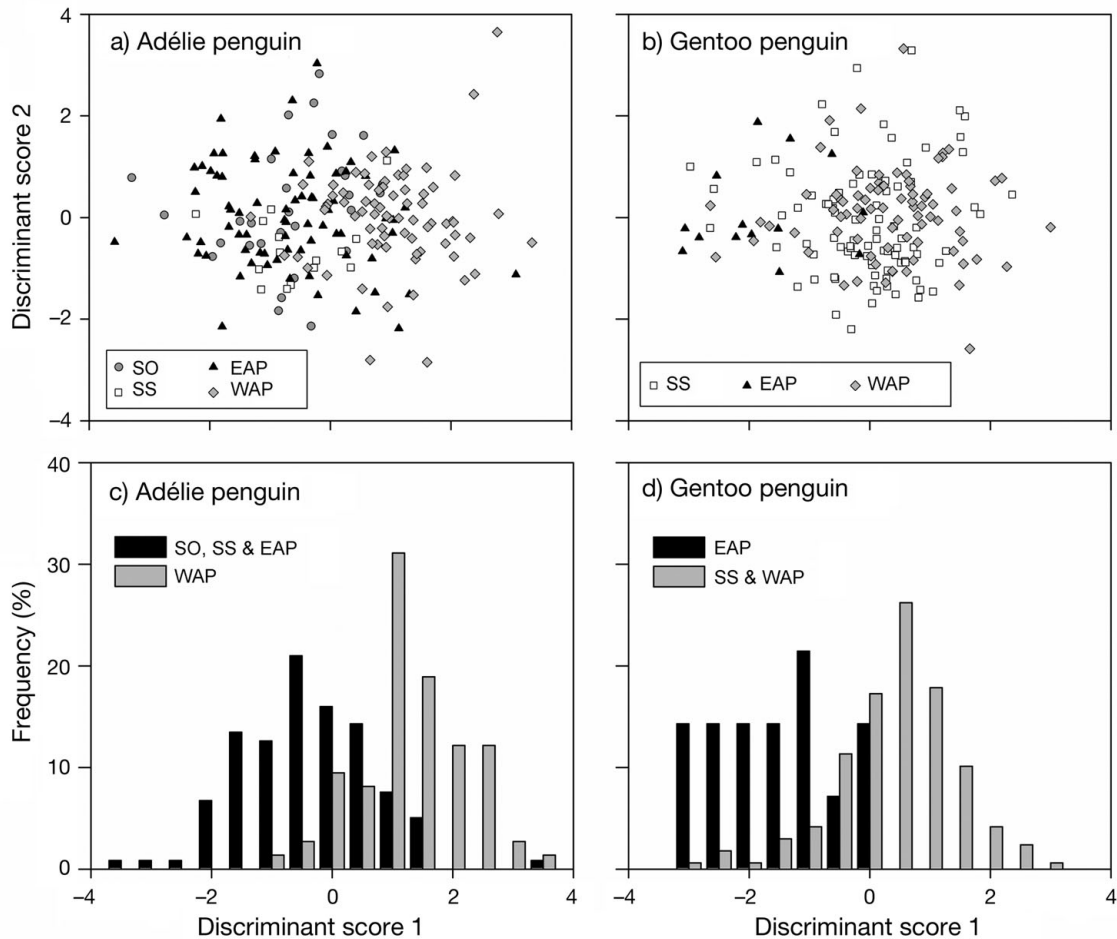


Fig. 3. *Pygoscelis adeliae* and *P. papua*. Discriminant analyses on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of eggshells collected from breeding colonies in the South Orkney Islands (SO), South Shetland Islands (SS), eastern Antarctic Peninsula (EAP), and western Antarctic Peninsula (WAP) during austral summer of 2006–07. Scatter plot of the 2 resulting discriminant functions for (a) Adélie and (b) gentoo penguin eggshells. Histograms of the single resulting discriminant function for (c) Adélie penguin eggshells from northern (SO, SS, EAP) and southern (WAP) regions and (d) gentoo penguin eggshells from eastern (EAP) and western (SS, WAP) regions

Table 1. *Pygoscelis adeliae* and *P. papua*. C:N ratio and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ concentrations (mean \pm SD) of eggshells collected from breeding colonies in 4 regions during austral summer of 2006–07. Groups that do not share at least 1 superscripted letter within a column are significantly different for the variable in question at the 0.05 level. n: no. of eggshells

Region, species	n	C:N	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
South Orkney Islands				
Adélie penguin	30	3.8 \pm 0.1	8.3 \pm 0.8 ^{a,b}	-24.8 \pm 0.5 ^a
South Shetland Islands				
Adélie penguin	15	3.7 \pm 0.2	7.9 \pm 0.5 ^a	-24.5 \pm 0.4 ^a
Gentoo penguin	88	3.8 \pm 0.2	8.7 \pm 0.8 ^{b,c}	-23.5 \pm 0.5 ^b
Eastern Antarctic Peninsula				
Adélie penguin	74	3.7 \pm 0.3	8.2 \pm 0.6 ^a	-24.8 \pm 0.6 ^a
Gentoo penguin	14	3.8 \pm 0.1	8.6 \pm 0.7 ^{a,b,c}	-24.5 \pm 0.5 ^a
Western Antarctic Peninsula				
Adélie penguin	74	3.6 \pm 0.2	8.9 \pm 0.6 ^c	-23.9 \pm 0.5 ^c
Gentoo penguin	80	3.9 \pm 0.2	8.9 \pm 0.7 ^c	-23.3 \pm 0.6 ^b

lambda = 0.792, $\chi^2 = 41.693$, df = 2, $p < 0.001$). Overall correct assignment after jackknife validation was 84.6% (Fig. 3d; 78.6% of the eastern group and 85.1% of the western group).

Prey items and pre-breeding diet composition

We found significant differences across the isotopic values of common Adélie and gentoo penguin prey species (Table 4; $\delta^{15}\text{N}$: $F_{2,60} = 637.78$, $p < 0.001$; $\delta^{13}\text{C}$: $F_{2,60} = 42.98$, $p < 0.001$). Post hoc Tukey comparisons determined that krill had significantly lower

Table 2. *Pygoscelis adeliae*. Location, C:N ratio, and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ concentrations (mean \pm SD) of eggshells collected from breeding colonies during austral summer of 2006–07. Groups that do not share at least 1 superscripted letter within a column are significantly different for the variable in question at the 0.05 level. Is. = Island; site no.: no. designating the respective site in Fig. 1; n: no. of eggshells

Region, site (site no.)	Latitude, longitude	n	C:N	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
South Orkney Islands					
Shingle Cove, Coronation Is. (1)	60.65° S, 45.57° W	15	3.7 \pm 0.1	7.9 \pm 0.6 ^a	−24.7 \pm 0.4 ^{a,b}
Gourlay Peninsula, Signy Is. (2)	60.72° S, 45.57° W	15	3.9 \pm 0.1	8.7 \pm 0.8 ^{b,c,d}	−24.9 \pm 0.6 ^a
South Shetland Islands					
Admiralty Bay, King George Is. (3)	62.17° S, 58.45° W	15	3.4 \pm 0.1	7.9 \pm 0.5 ^a	−24.5 \pm 0.4 ^{a,b,c}
Eastern Antarctic Peninsula					
Tay Head, Joinville Is. (9)	63.53° S, 56.92° W	14	3.9 \pm 0.1	8.0 \pm 0.5 ^a	24.4 \pm 0.7 ^{a,b,c}
Brown Bluff, Tabarin Peninsula (10)	63.35° S, 55.55° W	15	3.8 \pm 0.2	8.5 \pm 0.5 ^{a,b,c}	24.9 \pm 0.5 ^a
Paulet Is. (11)	63.58° S, 55.78° W	15	3.5 \pm 0.1	7.9 \pm 0.4 ^a	24.9 \pm 0.5 ^a
Devil Is. (12)	63.80° S, 57.28° W	15	3.8 \pm 0.2	8.4 \pm 0.8 ^{a,b,c}	24.7 \pm 0.5 ^{a,b}
Penguin Point, Seymour Is. (13)	64.30° S, 56.68° W	15	3.6 \pm 0.1	8.0 \pm 0.7 ^{a,b}	24.9 \pm 0.8 ^a
Western Antarctic Peninsula					
Dream Is. (15)	64.73° S, 64.23° W	14	3.7 \pm 0.1	8.9 \pm 0.3 ^{c,d}	24.1 \pm 0.5 ^{b,c,d}
Torgersen Is. (16)	64.77° S, 64.08° W	15	3.6 \pm 0.1	8.9 \pm 0.5 ^{c,d}	24.0 \pm 0.5 ^{cd}
Bisco Point, Anvers Is. (17)	64.80° S, 63.79° W	15	3.6 \pm 0.1	8.7 \pm 0.5 ^{b,c,d}	24.1 \pm 0.4 ^{b,c,d}
Yalour Is. (22)	65.23° S, 64.17° W	15	3.9 \pm 0.3	9.3 \pm 0.9 ^d	23.9 \pm 0.4 ^{c,d}
Berthelot Is. (23)	65.33° S, 64.15° W	15	3.6 \pm 0.1	8.7 \pm 0.5 ^{b,c,d}	23.7 \pm 0.4 ^d

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values relative to both fish species. The 2 fish species did not significantly differ in their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Table 4).

Using eggshell $\delta^{15}\text{N}$ values corrected with a discrimination factor derived from a captive population of gentoo penguins, our isotopic model estimated that fish comprised a significant portion (46.8 to 62.9%) of female Adélie and gentoo penguin diets prior to breeding (Table 5). Mixing-model estimates of the pre-breeding diets of female gentoo penguins did not differ significantly across regions (eastern AP

vs. South Shetland Islands: $t = 0.328$, $df = 45.888$, $p = 0.744$; eastern AP vs. western AP: $t = 0.981$, $df = 42.918$, $p = 0.332$; South Shetland Islands vs. western AP: $t = 0.775$, $df = 37.357$, $p = 0.443$). In contrast, our results suggest that female Adélie penguins breeding in the western AP consumed a larger proportion of fish during the pre-breeding period than did Adélie penguins from the other 3 regions (Table 5; South Orkney Islands vs. western AP: $t = 2.916$, $df = 68.451$, $p = 0.005$; South Shetland Islands vs. western AP: $t = 5.082$, $df = 36.8$, $p < 0.001$; eastern AP vs. western

Table 3. *Pygoscelis papua*. Location, C:N ratio, and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ concentrations (mean \pm SD) of eggshells collected from breeding colonies during austral summer of 2006–07. Groups that do not share at least 1 superscripted letter within a column are significantly different for the variable in question at the 0.05 level. Data for Cape Shirreff, Livingston Island are taken from Polito et al. (2009). Is. = Island; site no.: no. designating the respective site in Fig. 1; n: no. of eggshells

Region, site (site no.)	Latitude, longitude	n	C:N	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
South Shetland Islands					
Admiralty Bay, King George Is. (3)	62.17° S, 58.45° W	15	3.8 \pm 0.1	8.4 \pm 0.5 ^{a,b}	−24.1 \pm 0.6 ^a
Ardley Is., King George Is. (4)	62.22° S, 58.93° W	15	3.9 \pm 0.1	8.6 \pm 0.5 ^{a,b,c}	−23.4 \pm 0.4 ^{b,c}
Barton Peninsula, King George Is. (5)	62.24° S, 58.78° W	15	3.8 \pm 0.1	8.6 \pm 0.6 ^{a,b,c}	−23.2 \pm 0.4 ^{c,d}
Potter Peninsula, King George Is. (6)	62.25° S, 58.65° W	8	4.0 \pm 0.1	8.7 \pm 0.4 ^{a,b,c}	−23.1 \pm 0.5 ^{c,d}
Barrantos Is., Aitcho Is. (7)	62.41° S, 59.75° W	15	3.8 \pm 0.3	10.0 \pm 0.6 ^d	−23.5 \pm 0.5 ^{b,c}
Cape Shirreff, Livingston Is. (8)	62.47° S, 60.78° W	20	3.7 \pm 0.3	8.2 \pm 0.6 ^a	−23.4 \pm 0.3 ^{b,c}
Eastern Antarctic Peninsula					
Brown Bluff, Tabarin Peninsula (10)	63.35° S, 55.55° W	14	3.8 \pm 0.1	8.6 \pm 0.7 ^{a,b,c}	−24.5 \pm 0.5 ^a
Western Antarctic Peninsula					
Cuerville Is. (14)	64.68° S, 62.63° W	15	3.9 \pm 0.1	8.5 \pm 0.4 ^{a,b,c}	−23.9 \pm 0.5 ^{a,b}
Bisco Point, Anvers Is. (17)	64.80° S, 63.79° W	9	4.0 \pm 0.1	9.2 \pm 0.5 ^{b,c}	−23.5 \pm 0.3 ^{b,c}
Jouglia Point, Wiencke Is. (18)	64.82° S, 63.50° W	11	4.0 \pm 0.3	8.5 \pm 0.5 ^{a,b}	−23.4 \pm 0.5 ^{b,c}
Neko Harbour, Andvord Bay (19)	64.83° S, 62.55° W	15	3.9 \pm 0.2	9.1 \pm 0.8 ^{b,c}	−23.2 \pm 0.3 ^{c,d}
Pleneau Is. (20)	65.10° S, 64.07° W	15	3.8 \pm 0.1	8.8 \pm 0.6 ^{a,b,c}	−22.7 \pm 0.5 ^d
Petermann Is. (21)	65.17° S, 64.17° W	15	3.8 \pm 0.1	9.3 \pm 0.6 ^{c,d}	−23.2 \pm 0.3 ^c

Table 4. C:N ratio and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ concentrations (mean \pm SD) of common Adélie and gentoo penguin prey species collected during trawls conducted in the vicinity of the Antarctic Peninsula, South Shetland Islands, and South Orkney Islands during the austral summers of 2005–06 to 2008–09. Groups that do not share at least 1 superscripted letter within a column are significantly different for the variable in question at the 0.05 level

Prey type, species	n	C:N	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Krill				
<i>Euphausia superba</i>	20	3.7 \pm 0.2	3.2 \pm 0.7 ^a	-26.2 \pm 0.9 ^a
Fish				
<i>Pleuragramma antarcticum</i>	30	3.4 \pm 0.2	9.4 \pm 0.5 ^b	-24.7 \pm 0.4 ^b
<i>Lepidonotothen squamifrons</i>	10	3.3 \pm 0.1	9.6 \pm 0.8 ^b	-24.2 \pm 0.7 ^b

AP: $t = 4.331$, $df = 159.971$, $p < 0.001$). Furthermore, the results of our sensitivity analysis suggest that our estimates of the pre-breeding diets of female Adélie and gentoo penguins would change by 15.6 to 16.2% per 1‰ deviation from our assumed $\delta^{15}\text{N}$ discrimination factor of +1.8‰ (Fig. 4).

DISCUSSION

Habitat use prior to breeding

During the breeding season, the foraging ranges of Adélie and gentoo penguins are restricted to areas near their colonies (<100 km) due to the need to return regularly for incubation and chick-feeding duties (Trivelpiece et al. 1987, Clarke et al. 2006). During this time, Adélie penguins are generally shallow divers and forage in offshore, pelagic habitats, while gentoo penguins are found closer inshore, diving relatively deeper to forage in benthic habitats (Trivelpiece et al. 1987, Miller et al. 2009, Kokubun et al. 2010, Wilson

2010). We found that, in general, gentoo penguins had higher eggshell $\delta^{13}\text{C}$ values than Adélie penguins. Benthic and inshore marine food webs are more enriched in ^{13}C than pelagic and offshore food webs due to differences in carbon sources between the 2 habitats (benthic macro-algae vs. pelagic phytoplankton; France 1995, Dunton 2001). Our results suggest that, similar to during the breeding season, prior to breeding, female gentoo penguins feed more often in inshore, benthic habitats than Adélie penguins. These results agree

with previous observations that Adélie penguins inhabit the pack ice during winter, while gentoo penguins forage in open-water, near-shore habitats and remain close (<250 km) to their breeding colonies (Fraser et al. 1992, Wilson et al. 1998, Clausen & Pütz 2003, Tanton et al. 2004).

A possible exception to this generalization can be seen in the eastern AP, where eggshell $\delta^{13}\text{C}$ values did not differ between species. The single gentoo penguin colony sampled in the eastern AP (Brown Bluff, Tabarin Peninsula; Fig. 1) lies within Antarctic Sound, where many large tabular icebergs flowing westward from the Weddell Sea become grounded (Barker et al. 2007, M. Polito pers. obs.). Female gentoo penguins at Brown Bluff may be foraging more in pelagic habitats relative to gentoo penguins in other regions and/or $\delta^{13}\text{C}$ values may be lower in this region due to a reduction in benthic foraging habitats due to iceberg scour (Barnes 1999, Gerdes et al. 2003). While there may be regional variability in the types of foraging habitats used prior to breeding, it is likely that gentoo penguins in the AP generally forage in near-shore habitats.

Table 5. *Pygoscelis adeliae* and *P. papua*. Predicted diet compositions (mean \pm SE) and 95% confidence intervals (CI) of females during the pre-breeding period based on stable isotope analysis of eggshells collected from breeding colonies in 4 regions during austral summer of 2006–07. Estimates use the single isotope ($\delta^{15}\text{N}$), 2-source (krill and fish) mixing model described by Phillips & Gregg (2001), with eggshell $\delta^{15}\text{N}$ values corrected for isotopic discrimination (+1.8‰; Polito et al. 2009). Groups that do not share at least 1 superscripted letter within a column are significantly different for the variable in question at the 0.05 level

Region, species	n	Female pre-breeding diet composition (%)			
		Krill	95% CI	Fish	95% CI
South Orkney Islands					
Adélie penguin	30	46.8 \pm 2.7 ^{a,b,c}	41.2–52.3	53.2 \pm 2.7 ^{a,b,c}	47.7–58.8
South Shetland Islands					
Adélie penguin	15	53.2 \pm 2.6 ^a	48.0–58.5	46.8 \pm 2.6 ^a	41.5–52.0
Gentoo penguin	88	42.2 \pm 2.8 ^{b,c,d}	36.3–48.1	57.8 \pm 2.8 ^{b,c,d}	51.9–63.7
Eastern Antarctic Peninsula					
Adélie penguin	74	48.4 \pm 1.8 ^{a,b}	44.7–52.0	51.6 \pm 1.8 ^{a,b}	48.0–55.3
Gentoo penguin	14	43.8 \pm 3.8 ^{b,c,d}	35.9–51.6	56.3 \pm 3.8 ^{b,c,d}	48.4–64.1
Western Antarctic Peninsula					
Adélie penguin	74	37.1 \pm 1.9 ^d	33.4–40.8	62.9 \pm 1.9 ^d	59.2–66.6
Gentoo penguin	80	39.1 \pm 2.9 ^{c,d}	33.0–45.1	60.9 \pm 2.9 ^{c,d}	54.9–67.0

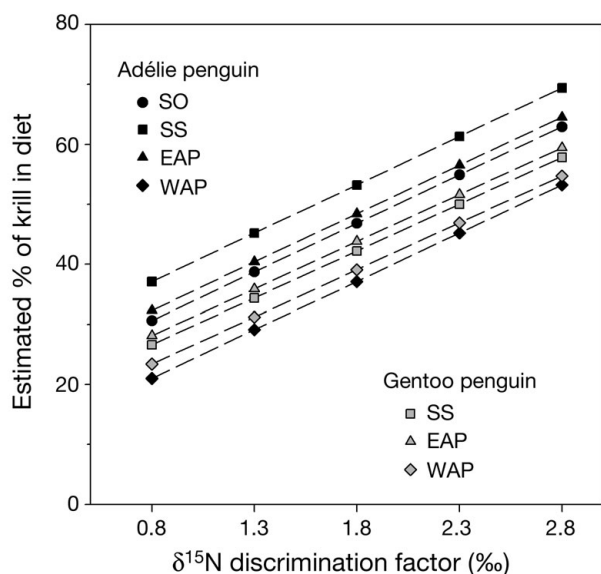


Fig. 4. *Pygoscelis adeliae* and *P. papua*. The effect of variation in $\delta^{15}\text{N}$ discrimination factor on the estimated diet composition (% of krill in diet) of females during the pre-breeding period based on stable isotope analysis of eggshells collected from breeding colonies in the South Orkney Islands (SO), South Shetland Islands (SS), eastern Antarctic Peninsula (EAP), and western Antarctic Peninsula (WAP) during austral summer of 2006–07. Estimates use the single isotope ($\delta^{15}\text{N}$), 2-source (krill and fish) mixing model described by Phillips & Gregg (2001). A $\delta^{15}\text{N}$ discrimination factor of +1.8‰ represents the value derived from a controlled dietary study of a captive population of gentoo penguins (Polito et al. 2009)

Therefore, variations in the stable isotope values of gentoo penguin eggshells within and across regions probably reflect local scale variation in the environmental conditions of their near-shore foraging grounds.

Distribution prior to breeding and the Adélie gap

In addition to delineating vertical trends (benthic vs. pelagic), natural isotopic patterns in the marine environment can infer the geographic origin of animal diets (reviewed in Hobson 1999). In the Southern Ocean, the $\delta^{13}\text{C}$ values of particulate organic matter track transitions between water mass sources defined by frontal zones and other oceanographic features with isotopic trends propagated up the food chain (Cherel & Hobson 2007, Phillips et al. 2009, Quillfeldt et al. 2010). When examining Adélie penguins breeding in the AP, we found that the isotopic signatures of their eggshells divided female penguins into 2 discrete groups: one north of the Adélie gap, comprised of breeding colonies in the South Orkney Islands, South Shetland Islands, and eastern AP, and the other

south of the Adélie gap, comprised of breeding colonies in the western AP. As shipboard survey data have shown that Adélie penguins are found almost exclusively in the pack ice outside of the breeding season, the observed isotopic differentiation between these 2 groups likely implies differences in their geographic location prior to breeding, rather than habitat type per se (Fraser et al. 1992, Chapman et al. 2004, Ribic et al. 2008). In addition, eggshells from breeding colonies to the south of the Adélie gap had higher $\delta^{13}\text{C}$ values than those to the north. As the $\delta^{13}\text{C}$ signatures of pelagic primary producers are higher in the Bellingshausen Sea relative to the Weddell Sea (Quillfeldt et al. 2010), our isotopic analyses provide support for the hypothesis that Adélie penguins that breed to the south of the Adélie gap (Anvers Island to the south along the western AP) winter in the Bellingshausen Sea and those that breed to the north of the Adélie gap (South Orkney Islands, South Shetland Islands, and eastern AP) winter in the Weddell Sea (Fraser et al. 1992, Fraser & Trivelpiece 1996, Trivelpiece & Fraser 1996).

Studies of Adélie penguins using devices such as satellite transmitters, GPS, and other data loggers suggest that after breeding, adults can travel as far as 17 000 km (though more commonly 1000 to 2000 km) away from their breeding colonies to reach the pack-ice zone where they overwinter (Kerry et al. 1995, Davis et al. 1996, 2001, Clarke et al. 2003, Ballard et al. 2010). In the AP region, recent tracking studies also provide support for our hypothesis of a common wintering range in the Weddell Sea for the 3 geographically disjunct Adélie penguin breeding groups found to the north of the Adélie gap. Dunn et al. (in press) tracked the winter movement of Adélie penguin breeding at Signy Island, South Orkney Islands and found that adults travel as far as 1300 km away from their breeding colony to winter in the pack ice of the Weddell Sea. In addition, adult Adélie penguins breeding on King George Island, South Shetland Islands, have been tracked to the pack ice in the eastern Weddell Sea, where they molt (W. Trivelpiece unpubl. data). Unfortunately, to our knowledge no published studies have documented winter movements of Adélie penguins breeding in colonies along the eastern or western AP. While tracking studies can provide detailed distributional data, small sample sizes and limited spatial (number of breeding colonies) and temporal (duration of tracking) scales make it difficult to extrapolate penguin distributional data at the population level. The present study highlights how the stable isotope analysis of penguin tissues can be used to confirm distributional trends identified by tracking and shipboard studies on a regional scale.

Diet prior to breeding

Most dietary studies of Adélie and gentoo penguins use stomach content analysis to examine diet composition during the chick-rearing period, when adults bring food ashore for their chicks. Studies in the AP suggest that during the breeding season, the diets of both species are often dominated by krill (when measured as wet mass), but that gentoo penguins also commonly consume fish (Trivelpiece et al. 1987, Lynnes et al. 2004, Miller et al. 2009). In contrast, our results suggest that high trophic-level prey such as fish comprised a significant portion (46.8 to 62.9%) of both female Adélie and gentoo penguin diets prior to breeding. This result agrees with the few existing studies of winter diets of Adélie and gentoo penguins in the AP region, which have found fish and squid to be important prey items for both species (Jablonski 1985, Ainley et al. 1992, Coria et al. 2000). While only the contributions of krill versus fish in penguin diets were assessed in our mixing-model analyses, it is important to note that as the fish and squid species consumed by *Pygoscelis* spp. penguins often have similar $\delta^{15}\text{N}$ values, the estimated fish portion of female penguins' pre-breeding diets likely represent the dietary contribution of all high trophic-level prey species such as fish and/or squid (Quillfeldt et al. 2005, Cherel 2008, M. Polito unpubl. data).

During the breeding season, gentoo penguins in the AP generally consume a relatively higher proportion of fish than Adélie penguins (Trivelpiece et al. 1987, Miller et al. 2009). However, inter-specific comparisons of diets prior to breeding are less clear. Overall mean gentoo penguin eggshell $\delta^{15}\text{N}$ values were higher than Adélie penguin values, and the range of $\delta^{15}\text{N}$ values observed at the breeding colony level was also higher in gentoo (8.4 to 10.0‰) than Adélie penguins (7.9 to 9.3‰). This result implies a relatively larger contribution of high trophic-level prey such as fish in gentoo penguin diets prior to breeding. However, when examining $\delta^{15}\text{N}$ values within regions, with the exception of the South Shetland Islands, $\delta^{15}\text{N}$ values did not vary by species. Furthermore, while mixing-model estimates of the proportion of fish (relative to krill) was generally higher in gentoo penguin diets, in some cases 95% confidence intervals overlapped between species (Table 5). These findings indicate that while gentoo penguins often consume a relatively higher proportion of fish than Adélie penguins prior to breeding, local and regional scale factors are also important determinants of pre-breeding diets.

Researchers have hypothesized that recent reductions in both krill recruitment and abundance affect Adélie and gentoo penguins differently, with the more krill-dependent Adélie penguins expected to be at a

relative disadvantage (Fraser et al. 1992, Smith et al. 1999, Forcada et al. 2006, Hinke et al. 2007). In addition, due to the similar breeding success of these 2 species, it has been hypothesized that their contrasting population trends are driven by factors operating outside the breeding season (Hinke et al. 2007, Carlini et al. 2009, Lynch et al. 2010). We found that the pre-breeding diets of female Adélie penguins in the AP, in contrast to diets during the breeding season, commonly included a large percentage of high trophic-level prey such as fish and/or squid. It is possible that seasonal shifts in the consumption of krill and fish and/or squid by *Pygoscelis* spp. penguins may be a reflection of the relative availability of these prey species in the marine environment. For example, a recent study examining Adélie penguin eggshells found compelling isotopic evidence of a shift from fish to krill in past penguin diets in conjunction with a proposed 'krill surplus' following the extensive harvest of whales and seals in the Southern Ocean (Emslie & Patterson 2007). Therefore, the reduced amount of krill and higher consumption of fish and/or squid in pre-breeding diets relative to the breeding season may be a reflection of the lower krill biomass available to penguins outside of the breeding season (Lascara et al. 1999).

Isotopic mixing model and discrimination factors

Isotopic mixing models are commonly used to determine the relative contribution of multiple food sources to animal diets in marine systems (Hammill et al. 2005, Becker et al. 2007, Navarro et al. 2009, Alves-Stanley et al. 2010). However, to our knowledge, other than a preliminary analysis of a portion of our own dataset (Polito et al. 2009), only one other study has used isotopic mixing-model procedures to estimate the diet composition of wild *Pygoscelis* spp. penguins. Tierney et al. (2008) compared mixing-model estimates of penguin diets based on the isotopic value of Adélie penguin chick blood to adult stomach contents collected during the chick-rearing period and found that these 2 methods provide similar estimates of penguin diets at a broad taxonomic level (i.e. krill vs. fish). Unfortunately, as stomach content analysis is often logistically impossible during the pre-breeding period, we do not have comparable data against which to validate our estimates of female pre-breeding diets, as done by Tierney et al. (2008).

Isotopic mixing models are based on geometric procedures that reconstruct animal diets based on the isotopic value of each food source after correcting for isotopic discrimination (the differences in isotopic ratios between food items and consumer tissues; Phillips & Gregg 2001, 2003, Post 2002, Caut et al.

2008b). Much of the uncertainty involved in the use of these methods derives from the selection of an appropriate discrimination factor, as models can be sensitive to variations in these values (Post 2002, Caut et al. 2008b). When estimating the composition of female Adélie and gentoo penguin diets prior to breeding, we corrected eggshell $\delta^{15}\text{N}$ values for dietary isotopic fractionation using a discrimination factor of +1.8‰ derived from a captive population of gentoo penguins (Polito et al. 2009). Eggshell $\delta^{15}\text{N}$ discrimination factors have only been quantified in 2 other avian species, quail and ostrich, with discrimination factors estimated at +1.0‰ to +3.0‰, respectively (Johnson 1995, Johnson et al. 1998). In addition to species-specific variation, other factors such as differences in physiological condition and the protein quality of diets between captive and wild populations could also bias discrimination factors and affect the accuracy of dietary reconstructions using isotopic mixing models (Hobson et al. 1993, Cherel et al. 2005a, Robbins et al. 2010).

It is for these reasons that we performed a sensitivity analysis to examine how resilient our estimates of female pre-breeding diets might be to variations in isotopic discrimination factors. We found that a 1‰ deviation in $\delta^{15}\text{N}$ discrimination factors resulted in a 15.6 to 16.2% change in our dietary estimates. Using an eggshell $\delta^{15}\text{N}$ discrimination factor near the upper limit of what has been described in previous studies (+2.8‰), fish comprised between 30.6 and 46.8% of estimated diet composition (Fig. 4). Our model results suggest that it would take a $\delta^{15}\text{N}$ discrimination factor >5.0‰ to result in an estimated diet composition that was comprised solely of krill. As this figure is well outside the range of eggshell discrimination factors currently described (reviewed in Polito et al. 2009), these findings, along with the few existing studies of winter diets, help to support our conclusion that high trophic-level prey such as fish and/or squid are important components of female Adélie and gentoo penguin diets prior to breeding.

CONCLUSIONS

The present study highlights the use of stable isotope analysis to provide insight into the diets and foraging habitats of female Adélie and gentoo penguins prior to breeding. Our results indicate that pre-breeding gentoo penguins feed more in benthic, inshore habitats relative to Adélie penguins, similar to differences observed during the chick-rearing period. However, unlike the breeding period when krill can dominate penguin diets, fish and/or other high trophic-level prey species comprise a significant portion of both female Adélie and gentoo penguin pre-breeding diets.

Finally, our findings confirm that Adélie penguin populations that are geographically separated during the breeding season by the Adélie gap also inhabit geographically distinct habitats prior to breeding. While our study examined the pre-breeding foraging ecology of Adélie and gentoo penguins over a large spatial scale, it is important to note the study's temporal limitations, as samples were collected during a single breeding season. Further work examining the isotopic signatures of eggshell is warranted to investigate how inter-annual variation in the pre-breeding diets and foraging habitats of Adélie and gentoo penguins may relate to breeding populations.

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